FORM PTO-1390 (REV 11-2000)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 1721-39

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): 09/980810 Unknown

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/01617	9 June 2000	10 June 1999

TITLE OF INVENTION
METHOD OF PRODUCTION OF COMPLEX MIXTURES OF cDNA AND APPLICATIONS OF THESE MIXTURES FOR THE ANALYSIS OF GENE EXPRESSION

APPLICANT(S) FOR DO/EO/US
ARNOULD et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The U.S. has been elected by the expiration of 19 months from the priority date (Article 31).
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   - b. ☒ has been communicated by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a. ☒ is attached hereto.
   - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   - b. ☐ have been communicated by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
   - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☒ A English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 To 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information. PTO-1449 and copy of International Search Report

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
Unknown 09/979810	PCT/FR00/01617	1721-39

21. ☒ The following fees are submitted:

	CALCULATIONS	PTO USE ONLY

BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):

-- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1040.00

-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $890.00

-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO $740.00

-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $710.00

-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =						$	890.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).						$	130.00	
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE				
Total Claims	6	-20 =	0	X	$18.00	$	0.00	
Independent Claims	1	-3 =	0	X	$84.00		0.00	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)					$280.00	$	0.00	
TOTAL OF ABOVE CALCULATIONS =						$	**1020.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.							0.00	
SUBTOTAL =						$	**1020.00**	
Processing fee of $130.00, for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)). +							0.00	
TOTAL NATIONAL FEE =						$	**1020.00**	
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). **$40.00** per property +						$	0.00	
Fee for Petition to Revive Unintentionally Abandoned Application ($1280.00 – Small Entity = $640.00)						$	0.00	
TOTAL FEES ENCLOSED =						$	**1020.00**	
						Amount to be: refunded	$	
						Charged	$	

a. ☒ A check in the amount of $1020.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 14-1140 in the amount of $_____ to cover the above fees. A duplicate copy of this form is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed.

d. ☒ The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

B. J. Sadoff
NAME

36,663 **December 6, 2001**
REGISTRATION NUMBER Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

ARNOULD et al Atty. Ref.: **1721-39**

Serial No. **Unknown** Group:

National Phase of: **PCT/FR00/01617**
International Filing Date: **9 June 2000**

Filed: **Herewith** Examiner:

For: **METHOD OF PRODUCTION OF COMPLEX MIXTURES OF CDNA AND APPLICATIONS OF THESE MIXTURES FOR THE ANALYSIS OF GENE EXPRESSION**

* * * * * * * * * * * *

December 6, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to calculation of the filing fee and in order to place the above identified application in better condition for examination, please amend as follows:

IN THE SPECIFICATION

Page 1, after the title insert the following:

-- This application is the US national phase of international application PCT/FR00/01617 filed June 9, 2000 which designated the U.S. --.

IN THE CLAIMS (AS PER IPER)

Please substitute the following amended claims for corresponding claims previously presented. A copy of the amended claims showing current revisions is attached.

3. (Amended) Kits for the synthesis of cDNA mixtures according to the method of Claim 1, characterized in that they contain, in addition, reagents for carrying out

reverse transcription, elongation terminators, especially dideoxynucleotides, and instructions for use.

4. (Amended) cDNA mixtures as obtained by application of the method according to Claim 1, reliably reflecting the state of transcription of a tissue or of cells, i.e. the number of and the level of gene expression.

REMARKS

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**."

The above amendments are made to place the claims in a more traditional format.

Respectfully submitted,
NIXON & VANDERHYE P.C.
By: _____
B. J. Sadoff
Reg. No. **36,663**

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

3. (Amended) Kits for the synthesis of cDNA mixtures according to the method of Claim 1 [or 2], characterized in that they contain, in addition, reagents for carrying out reverse transcription, elongation terminators, especially dideoxynucleotides, and instructions for use.

4. (Amended) cDNA mixtures as obtained by application of the method according to Claim 1 [or 2], reliably reflecting the state of transcription of a tissue or of cells, i.e. the number of and the level of gene expression.

21 pts

METHOD OF PRODUCTION OF COMPLEX MIXTURES OF cDNA AND APPLICATIONS OF THESE MIXTURES FOR THE ANALYSIS OF GENE EXPRESSION

The invention relates to a method for the production of complex mixtures of cDNA and the applications of the said mixtures, in particular as probes for studying profiles of gene expression in a tissue or cells of animal, plant or microbial origin.

The methods that have been developed for collecting the expression profiles of a large number of genes are based on the hybridization of complex cDNA probes derived from messenger RNAs (mRNA's) on various substrates carrying either cDNA clones, or specific oligonucleotides of thousands of genes.

Obtaining cDNA's by reverse transcription (RT) of mRNA's comprises, conventionally, the use of, as primers, either oligo (dT), or of randomly synthesized oligonucleotides, or of specific oligonucleotides of the genes studied. In the first case, the oligo(dT) is attached to the poly(A) tail of the mRNA's (which are characteristic of the 3'OH end of eukaryotic messenger RNAs) and reverse transcriptase extends the oligo(dT) primer in the direction of the 5'P end of the mRNA's. In the second case, the primer, consisting of oligonucleotides synthesized at random, is attached randomly on the whole length of the mRNA and elongation of the cDNA is effected in the same conditions as those described above. In the 3rd case,

attachment is effected specifically on the genes being studied, and elongation is effected as described previously.

Using these cDNA's as probes, it is a question of simultaneously obtaining several thousands of hybridization signals which reflect biological situations and their dynamic variations for studies of physiology, pathology, or pharmacology, as well as for comparative studies of various model organisms.

It will be appreciated that the reliability of these studies depends on the nature of the cDNA mixture produced, which must reflect the complexity of the mRNA population regardless of the level of abundance of the molecular species of which it is constituted.

However, the technique cited above, which is the one most commonly used in laboratories, and the kits that are being marketed for cDNA synthesis by the two types of reaction indicated above, do not permit satisfactory transcription of the whole of an mRNA population, and especially of the mRNA's that are poorly represented.

In the standard technique of reverse transcription, each molecule of reverse transcriptase initiates the synthesis of a complementary chain of an mRNA, then stops spontaneously after elongation of several hundred nucleotides, and detaches itself from the chain in the course of synthesis. It then attaches itself to another chain in the course of synthesis and continues its elongation. This has the effect of favouring the most abundant mRNA's in the starting sample.

A method of gene cloning in which the mRNA corresponding to the gene is present in very small proportions in a mixture of mRNA is described in patent US 4 738928.

A short amino acid sequence of the required peptide (5 to 25 amino acids) must be known. This method then envisages preparing a complementary primer which is used in a reverse transcription process in an mRNA mixture containing the target. Chain terminators are used for stopping elongation.

In the article of Koch G. and Kant A. in Nucleic Acids Research, Vol. 18, No. 10, 1990, p. 3063-3065, the authors undertake the synthesis, in the conventional manner, of cDNA starting from genomic RNA, employing RT in a method of chain termination by dideoxy.

The inventors found that this technique could be improved by controlling transcription.

The aim of the invention is therefore to provide a method of producing very reliable and reproducible cDNA mixtures.

It also relates to these mixtures as such and their uses especially as hybridization probes.

The method according to the invention, for the production of a complex mixture of cDNA by reverse transcription of mRNA from tissues or cells, is characterized by the addition of elongation terminators in the reaction mixture, recovery of the cDNA mixture formed, preferably followed by its purification.

Surprisingly, the addition of elongation terminators has the effect of preventing the re-initiation mentioned above, and the molecules of reverse transcriptase then initiate the synthesis of complementary chains of the mRNA molecules that are poorly represented (the least abundant). As a result, the complex mixtures of cDNA produced in the presence of elongation terminators represent all of the starting mRNA, including the mRNA's present in small amounts.

The mRNA employed in the method of the invention is obtained from cells in culture or from samples or from tissue, and can be of any origin: animal, plant or microbial.

An elongation terminator that is widely used consists of dideoxynucleotides.

The reaction of reverse transcription is carried out in particular in accordance with the usual techniques.

Synthetic oligonucleotides, such as are employed conventionally in RT techniques, will be used advantageously as elongation primers. Oligonucleotides that are suitable for implementation of the method of the invention include hexamers or oligonucleotides synthesized at random. Labelling means are preferably added to the reaction medium, for example radioactive elements, fluorescent, luminescent or colorimetric agents, to provide labelled cDNA's for subsequent applications.

In general, the method according to the invention is applicable for all operations of production of cDNA by RT starting from the mRNA from a tissue or from a cell, of whatever origin.

It has the advantage of being highly reproducible, reliable, efficient, and does not give rise to a notable extra cost. The yield of the transcription reaction can reach 90% or even exceed this value, whereas it generally only reaches about 30% in procedures based on the usual conditions.

As shown in the examples, this method has the advantage of making it possible to study a very large number of genes and their levels of expression, whatever the species or tissue studied, as it is able to reveal genes that are poorly expressed.

The levels of gene expression can then be determined very reliably by counting cDNA's that have been cloned and sequenced, for example by the SAGE method or partial sequencing of cDNA libraries.

The invention also relates to kits for the application of the aforementioned method for purposes of synthesis of the said cDNA mixtures. These kits are characterized in that they include, in addition, reagents for carrying out reverse transcription, elongation terminators, in particular dideoxynucleotides, and instructions for use.

The invention relates to complex cDNA mixtures, as novel products, such as are obtained by the method defined above and using the said kits if necessary.

These mixtures are characterized in that they reliably reflect the state of transcription of a tissue or of cells, i.e. the number and the level of gene expression.

The said mixtures therefore constitute high-quality copies of transcriptons and thus make it possible to improve, in hybridization experiments, the performance of complex cDNA probes produced starting from these mixtures.

The invention therefore also relates to the use of complex cDNA mixtures as hybridization probes.

Advantageously, these probes make it possible to detect the expression of a large number of genes by greatly improving the capacity for detecting the activity of poorly expressed genes.

The quality of the cDNA's of the complex mixture, as probes, was tested by synthesizing complex probes starting from messenger RNAs from various tissues.

By using high-density filters, which make it possible to study the hybridization, simultaneously, of a large number of clones, the inventors demonstrated that the complex mixtures according to the invention made it possible to detect, on the filters, a number of clones 50 to 150% greater than is observed with the cDNA mixtures employed until now, the majority of the clones thus identified being clones corresponding to poorly expressed genes.

The invention therefore also relates to a method of studying the profile of gene expression in a tissue or in cells.

The said method comprises bringing the labelled cDNA mixtures defined above into contact with the DNA to be investigated (cDNA, or specific oligonucleotides of DNAs), in conditions permitting the hybridization of the complementary sequences when they are present.

The procedure is carried out in stringent or in non-stringent conditions, with substrates on which the DNAs to be studied are deposited.

These supports can be Nylon® filters, or other supports as well, including sheets of glass.

Suitable conditions correspond to the use of a temperature of 68°C for 2 hours with the same solution with 20 x 10^6 cpm of probe.

The filters are washed, and then the hybrids that have formed are revealed.

The invention thus provides means for studying gene expression and the variations of this expression as a function of certain factors.

Accordingly, it makes it possible to identify targets at the therapeutic level for drug development.

Examples that may be given are applications in oncology, chiefly in cancers of the colon, for identifying the genes that are altered in the cancerous cells relative to the healthy cells and for determining potential targets for development of therapies; in neuromuscular diseases for identifications of the aforementioned type in muscle, for example in Duchenne's muscular dystrophy (DMD); for carrying out studies on muscles in conditions of weightlessness, or in neurodegenerative diseases (Parkinson's disease or amyotrophic lateral sclerosis (ALS)).

Other applications in cattle and goats have the purpose of studying the modified genes in the mammary gland during gestation and lactation, in order to find targets for improving milk quality and quantity.

Other characteristics and advantages of the invention will be apparent from the examples given below, in which reference is made to Figs. 1 and 2, showing respectively, according to the four conditions of initiation of RT,

- Figure 1, the ratio between the size of the inserts in kb and the rates of hybridization,

- Figure 2A, the hybridization of high-density filters with complex cDNA probes derived from poly(A)$^-$ mRNA from murine skeletal muscle, and

- Figure 2B, the distribution of the clones according to type of hybridization.

Example 1: Production of a complex mixture of cDNA from mRNA of murine skeletal muscle.

The results of 5 series of experiments are given.

Each time, a complex mixture of cDNA is synthesized by reverse transcription of 500 ng of poly(A)$^-$ mRNA from murine skeletal muscle. The reaction is carried out using the SuperScript® pre-amplification system for synthesizing the first strand of cDNA (Life Technologies SARL, Cergy Pontoise, France), following the manufacturer's recommendations. The following are used: 500 ng of hexamers or 500 ng of oligo(dT) primers, 50 µCi of [α-^{33}P]dATP, 3000 Ci/mmol (Amersham France, S.A., Les Ulis, France) and 500 µM of d(T, C, G)TP (Pharmacia Biotech, Orsay, France), in a final volume of 50 µl.

The complex mixture obtained is purified on a column of Sephadex G-50® (Quick Spin®, Boehringer Mannheim, France S.A., Meylan, France).

The amount of radioactivity before and after purification is determined using a scintillation counter (Beckman Instruments France S.A., Gagny, France) for calculating the specific activity of the complex cDNA mixture (cpm/µg of cDNA) and the cDNA yield in the synthesis (%). The cDNA yield in the synthesis is calculated using a theoretical maximum of cDNA synthesis equal to 20 ng, a value that arises from the limiting concentration of dATP in the reaction (0.3 µM).

The following table gives the ratio between the conditions of initiation of the RT reaction, the specific activity of the complex cDNA mixtures (SA, cpm/µg of cDNA) and the cDNA yield in synthesis (% of the theoretical maximum).

Oligo(dT)		Oligo (dT) + ddTTP		Hexamers		Hexamers + ddTTP	
SA	Yield	SA	Yield	SA	Yield	SA	Yield
*3.10^9	44	6.10^9	28	5.10^9	1	5.10^9	100
3.10^9	15	6.10^9	31	5.10^9	21	3.10^9	100
2.10^9	17	‘5.10^9	38	5.10^9	19	5.10^9	95
4.10^9	42	5.10^9	39	*3.10^9	24	*5.10^9	89
4.10^9	89	5.10^9	39	3.10^9	9	5.10^9	79

"*" corresponds to the probes used for the hybridizations of filters shown in Figure 2.

Examination of this table shows that the specific activity of the complex cDNA mixtures is in the range from 2 to 6 x 10^9 cpm/µg regardless of the conditions of RT initiation. Using oligo(dT) primers or hexamers, the yield of the RT reaction, which ranges from 15 to 89% and from 1 to 24% respectively, seems very variable.

On the other hand, in the presence of dideoxynucleotides, the average yield of the reaction, equal to about 35% using oligo(dT) and to about 95% using hexamers, seems much more reproducible.

The data in this table also shows that the incorporation of dideoxynucleotides in the reaction mixture improves the efficiency of cDNA synthesis initiated with hexamers by a factor of 5 on average with all the values above 79%. Similar results are obtained working with 50 and 5 µM of ddTTP (i.e. 1/10 or 1/100 of dNTP concentration) and with ddCTP instead of ddTTP.

Example 2: Use of the complex cDNA mixture from Example 1 as a probe.

The purified complex cDNA mixture is used for purposes of hybridization on high-density Nylon® filters (Hybond-N+, Amersham France S.A., France), comprising the products of PCR amplification of the inserts of 1339 DNA clones from a bank of human skeletal muscle.

The filters are pre-hybridized at 68°C for 30 minutes in an ExpressHyb® hybridization solution (Clontech Inc., Palo Alto, CA, USA). Hybridization is carried out at 68°C for 2 hours in the same solution with 20 x 10^6 cpm of probe. The filters are washed twice at room temperature for 30 minutes in SSC 1X/0.1% of SDS, and twice for 30 minutes in SSC 0.1X/0.1% of SDS.

These non-stringent conditions permit optimum hybridization between the murine cDNA probes and the human cDNA targets. The filters are then exposed to phosphorus screens for 16 hours (Molecular Dynamics S.A., Paris, France).

For each filter, the 1339 hybridization signals are identified and quantified using software specially designed for this application (XdotReader, Cose, France). For each clone, the value of the rate of hybridization is calculated as described in Piétu et al., Genome Research, 1996, 6:492-503, and standardized by dividing by the mean of all the intensity values on each filter.

The use of hexamers and/or dideoxynucleotides in the RT reaction does not introduce any deviation in the hybridization of cDNA inserts of different lengths, as is shown by the absence of overall correlation between signal intensity and the length of the cDNA inserts ($r \leq 0.05$ with the four conditions of initiation tested, presented in Figure 1).

Figure 2A illustrates the qualitative aspects of the hybridization of high-density filters with the complex cDNA probes obtained in each condition tested.

Analysis of the hybridization signals enables us to assign intensity values to the 539 (40%), 451 (33%), 797 (60%) and 1122 (83%) clones, which differ from the background noise when the probe is primed with, respectively, oligo(dT), hexamers, oligo(dT) + ddTTP and hexamers + ddTTP.

These results show an overall increase of the clones detected in the presence of dideoxynucleotides: +47% and +148% using oligo(dT) primers or hexamers, respectively.

Figure 2B shows quantitative analysis of the values of hybridization rate distributed in four representative categories: background noise (B), weak (f), medium (M) : 2 times the weak value, and strong (F): 6 times the weak value. The symbols used have the following meanings: oligo(dT) primers: ■ , hexamers: ⊠ , oligo(dT) + ddTTP primers: □ , and hexamers + ddTTP: □ . The number of clones in each category of hybridization intensity is shown at the top of each histogram.

The number of clones with strong or medium values of hybridization rates shows limited variations when the four RT conditions are used.

On the contrary, the number of clones with weak intensity values is more than doubled when using the dideoxynucleotides with the oligo(dT) primers, and

multiplied nearly 18 times when they are used with hexamers. In these conditions the fraction of clones associated with the hybridization signals that cannot be distinguished from the background noise with a high reliability is limited to 16%, which illustrates the improved ability of the corresponding complex cDNA probe to reflect the least abundant transcripts.

Furthermore, the values of hybridization rates obtained with a complex cDNA probe produced with the hexamers and the dideoxynucleotides in 4 hybridizations of the same high-density filter differs by -10%.

CLAIMS

1. A method of obtaining a transcripton comprising the production of a complex cDNA mixture by reverse transcription of mRNA from tissues or cells, characterized by the addition, in the mRNA reaction medium, of elongation terminators, recovery of the cDNA mixture formed, preferably followed by its purification.

2. A method according to Claim 1, characterized by the use of dideoxynucleotides as elongation terminators.

3. Kits for the synthesis of cDNA mixtures according to the method of Claim 1 or 2, characterized in that they contain, in addition, reagents for carrying out reverse transcription, elongation terminators, especially dideoxynucleotides, and instructions for use.

4. cDNA mixtures as obtained by application of the method according to Claim 1 or 2, reliably reflecting the state of transcription of a tissue or of cells, i.e. the number and the level of gene expression.

5. Use of complex cDNA mixtures according to Claim 4 as hybridization probes on high-density filters.

6. A method for studying the profiles of expression of the genes present in a tissue or in cells, characterized in that it comprises bringing cDNA mixtures according to Claim 4 into contact with the DNA to be studied

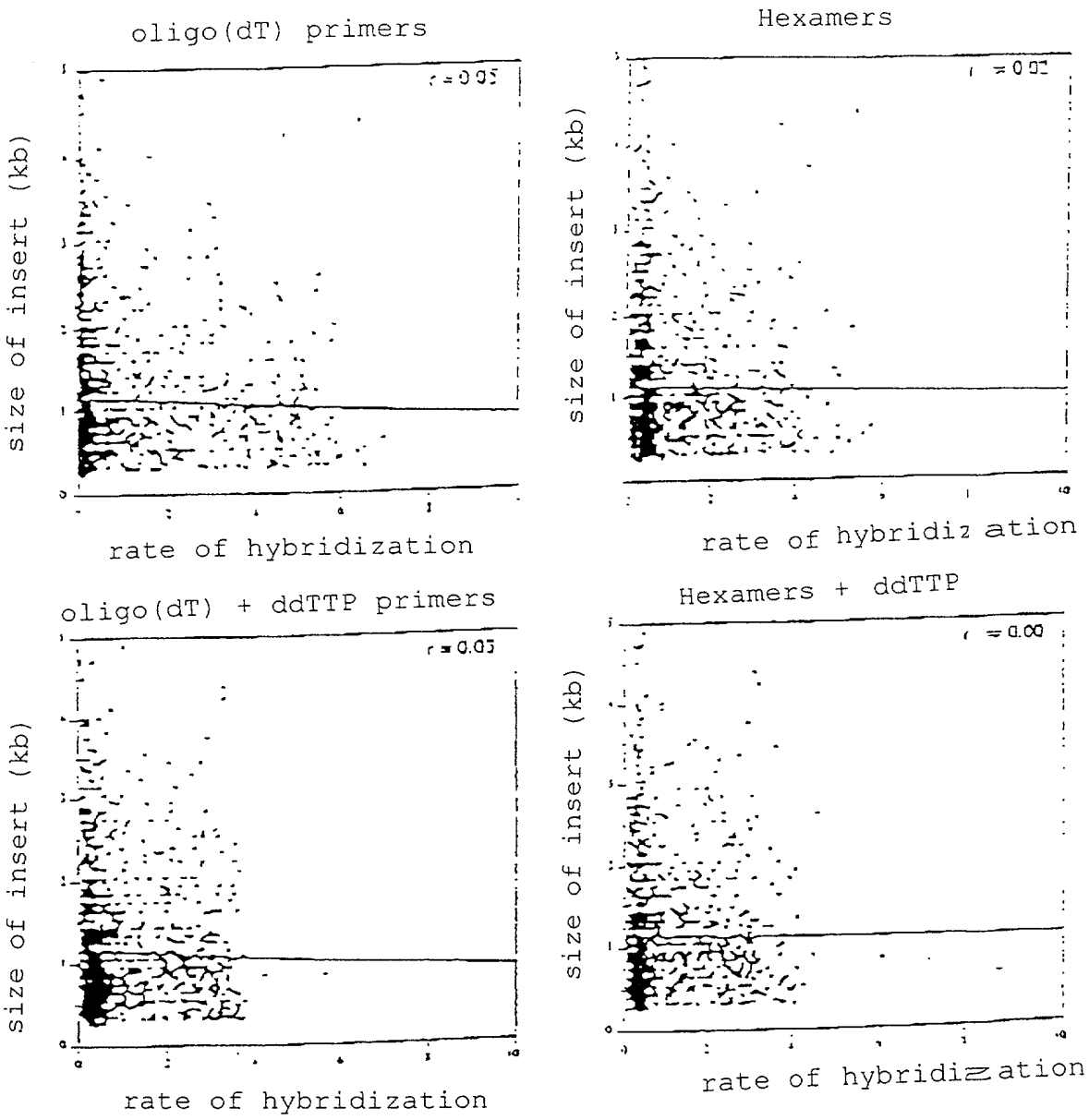
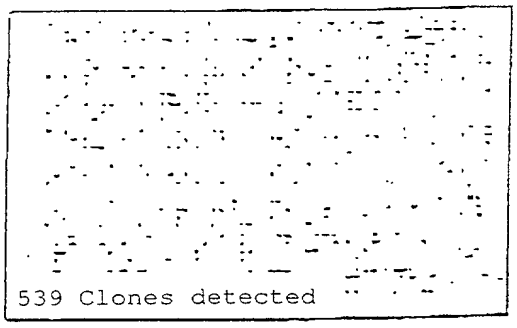

oligo(dT) primers
Hexamers
oligo(dT) + ddTTP primers
Hexamers + ddTTP
size of insert (kb)
rate of hybridization

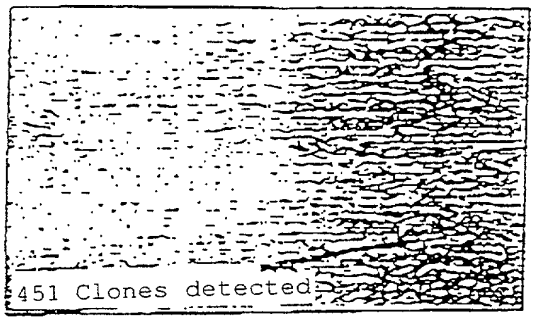
FIGURE 1

FIGURE 2

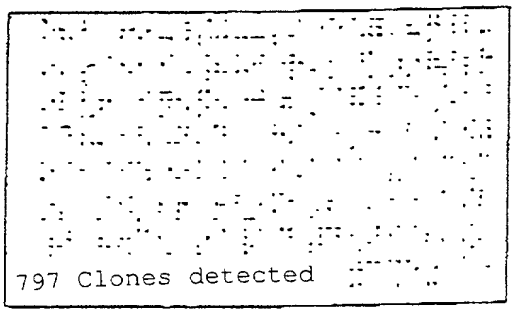
2 (A) oligo(dT) primers



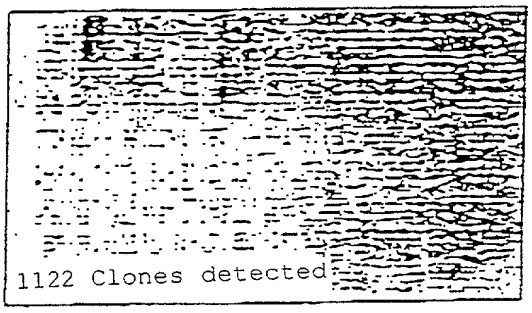
Hexamers



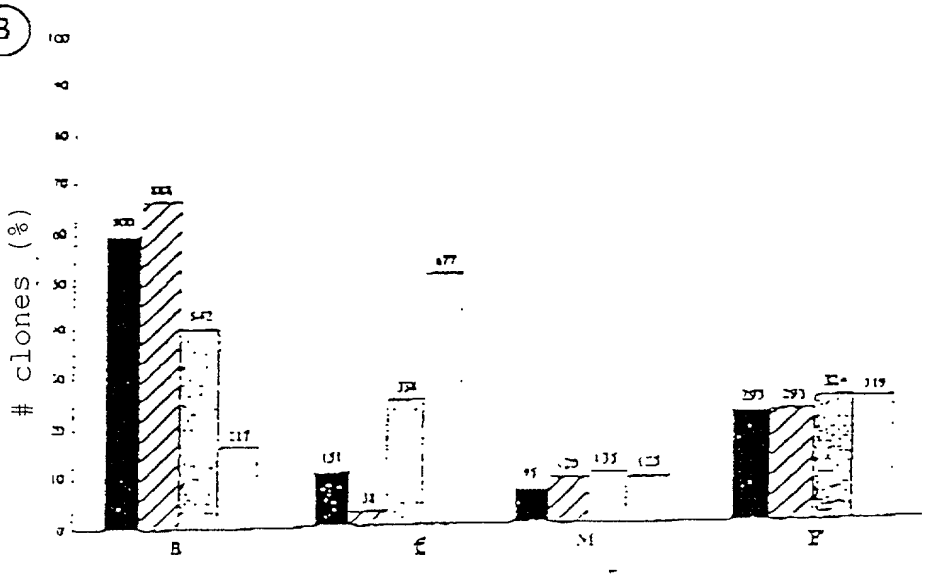
oligo(dT) + ddTTP primers



Hexamers + ddTTP



2 (B)



Nixon & Vanderhye P.C. (6/92)

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: Method for producing complex mixtures of cDNA and uses of said mixtures for gene expression analysis.

the specification of which (check applicable box(s)):

- [ ] is attached hereto.
- [ ] was filed on _____ as U. S. Application Serial No. _____
- [X] was filed as PCT international application No. PCT/ FR00/01617 on June 9, 2000

and (if applicable to U.S. or PCT application) was amended on Art.39 July 16, 2001

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Prior Foreign Application(s):

Application Number	Country	Day/Month/Year Filed
99 07357	FR	10 June 1999

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT international applications listed above or below and, insofar as the subject matter of each of the claims of this application is not disclosed in such prior applications in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. 1.56 which occurred between the filing date of the prior applications and the national or PCT international filing date of this application:

Prior U.S./PCT Application(s):

Application Serial No.	Day/Month/Year Filed	Status: patented, pending, abandoned
PCT/FR00/01617	9 June 2000	pending

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And I hereby appoint **NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed)**, and the following attorneys thereof (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Arthur R. Crawford, 25327; Larry S. Nixon, 25640; Robert A. Vanderhye, 27076; James T. Hosmer, 30184; Robert W. Faris, 31352, Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 33363; Paul J. Henon, 33626, Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr., 29366; Thomas E. Byrne, 32205; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Jerry D. Craig, 38026.

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